REMARKS

This paper is filed in Response to the final Office Action mailed July 20, 2007. Claims 1, 2, 4, 7 to 16 and 54 to 65 are pending and under consideration.

Regarding the Claim Amendments

The amendments to the claims are supported throughout the specification or were made to address an informality. In particular, the amendments to claims 9, 10 and 11 to recite that the functional fragment specifically binds to at least one of the recited cell lines is supported, for example, by claims 1, 4, 6 and 9 to 11 and 14, as originally filed. The amendment to claim 11 to depend from claim 1 was made in order to reduce the number of independent claims. Thus, as the claim amendments are supported by the specification or were made to address an informality, no new matter has been added. The amendments to the claims also do not raise new issues and place the claims in better form for allowance or appeal. Accordingly, entry of the claim amendments is respectfully requested.

I. REJECTION UNDER 35 U.S.C. §112

The rejection of claims 1, 2, 4, 7 to 11, 14, 15 and 54 to 65 under 35 U.S.C. §112, first paragraph as allegedly lacking enablement is respectfully traversed. According to the Patent Office, allegedly the specification does not enable the skilled artisan to make and use the invention commensurate in scope with the claims.

The proper standard for enablement under 35 U.S.C. §112, is whether one skilled in the art could make and use the invention without undue experimentation. In this regard, "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands* 858 F.2d 731, 737 (Fed. Cir. 1988).

Here, in view of the guidance in the specification and knowledge in the art regarding antibody structure and function at the time of the invention, and that antibody variants and functional fragments having the requisite activity could be produced and identified using routine methods disclosed in the specification or that were known in the art at the time of the invention, one skilled in the art could make and use the claimed antibodies and functional fragments comprising a sequence at least 85% identical to the amino acid sequence of SEQ ID NO:1 and a

sequence at least 85% identical to the amino acid sequence of SEQ ID NO:3, and heavy and light chain sequences of SEQ ID NO:1 and SEQ ID NO:3, without undue experimentation.

As pointed out in Applicants previous Response, the level of knowledge with respect to antibody structure and function was high at the time of the invention. In particular, knowledge regarding antibody structure and function, such as native antibodies having two heavy and light chain sequence, the presence and contribution of three CDRs to binding, and the role of framework regions (FRs) was acknowledged by the Examiner in the Office Action mailed January 3, 2007 (see pages 5 and 6). The specification discloses the function of antibody heavy and light chain variable regions (page 17, line 28, to page 18, line 20). The role of antibody heavy and light chain variable regions, particularly CDRs and FRs, in antigen binding were also well understood by the skilled artisan at the time of the invention. Consequently, the level of knowledge in the art with respect to antibody structure and function at the time of the invention was high.

Because the level of knowledge in the art with respect to antibody structure and function was high at the time of the invention, such as the sequences that contribute to antigen binding of antibodies (e.g., CDRs and FRs), the skilled artisan would know residues of SEQ ID NO:1 and SEQ ID NO:3 that would be amenable to substitution and would therefore be able to predict with reasonable certainty antibody variants of SEQ ID NO:1 and SEQ ID NO:3 that would have at least partial cell binding activity. As a non-limiting example illustrating this point, the skilled artisan would know that an amino acid substitution, such as a conservative substitution, of SEQ ID NO:1 or SEQ ID NO:3, particularly a conservative substitution outside of a CDR or FR region would likely not destroy binding activity. Thus, the skilled artisan could make a conservative amino acid substitution outside of a CDR or FR with a reasonable certainty that the substituted sequence would retain at least partial activity of non-substituted sequence. Given the large number of amino residues outside the CDR and FR regions, as well as the large number of amino residues outside of antibody variable regions, clearly many variants produced would have at least partial cell binding activity of non-variant SEQ ID NO:1 or SEQ ID NO:3. As an additional non-limiting example illustrating this point, the skilled artisan would know that given the contribution of CDRs to antigen binding a large number of non-conservative amino acid substitutions in the hypervariable region of SEQ ID NO:1 or SEQ ID NO:3 would likely reduce or eliminate binding. Thus, the skilled artisan would know not to introduce a large number of

non-conservative substitutions or delete a large number of amino acids into SEQ ID NO:1 or SEQ ID NO:3. Consequently, in view of the guidance in the specification and the high level of knowledge in the art regarding antibody structure and function, the skilled artisan would know of general regions and particular residues that would be more or less amenable to substitution and could therefore predict variants and fragments that are likely to have at least partial function of non-variant sequence without actually producing variants and fragments.

In addition to knowing regions and residues that would be more or less amenable to substitution or deletion, the level of skill in the art regarding producing antibodies and functional fragments thereof was also high. For example, conventional methods of producing antibody variants without undue experimentation are disclosed in the specification (page 22, line 16, to page 24, line 9; and page 21, line 16, to page 22, line 14). Such methods include conservative amino acid substitutions at pre-determined locations (page 23, line 23, to page 24, line 9). Furthermore, methods of producing antibody fragments (*e.g.*, Fv, Fab, Fab' and $F(ab')_2$) were known in the art and were routine at the time of the invention (*e.g.*, using recombinant techniques). Methods of identifying which antibody variants and fragments have the recited cell binding and other activities without undue experimentation are also taught by the specification and were also known in the art at the time of the invention. In particular, methods for measuring antibody binding to the recited cell lines and ascertaining cell proliferation and apoptosis are disclosed in the specification (page 44, Example 3, to page 49, Example 6). Thus, in view of the guidance in the specification and the high level of skill in the art at the time of the invention regarding producing antibodies and functional fragments, one skilled in the art could make and use the claimed antibodies and functional fragments comprising a sequence at least 85% identical to the amino acid sequence of SEQ ID NO:1 and a sequence at least 85% identical to the amino acid sequence of SEQ ID NO:3, and heavy and light chain sequences of SEQ ID NO:1 and SEQ ID NO:3, without undue experimentation.

Moreover, Applicants also respectfully point out that if the skilled artisan wished to produce antibody variants and functional fragments, producing recombinant proteins was routine in the art at the time of the invention, and the specification discloses routine assays for identifying antibodies that bind to the recited cell types, as well as cell proliferation/apoptosis assays. Analogous to *In re Wands,* 858 F.2d 731 (Fed. Cir. 1988), where the court held that screening hybridomas to determine those that produced monoclonal antibodies having a

particular binding characteristic did not require undue experimentation, given that 1) producing antibody variants and fragments was routine at the time of the invention; and 2) cell binding assays were routine at the time of the invention, undue experimentation would not be required to identify which antibody variants and fragments of SEQ ID NO:1 and SEQ ID NO:3 bind to the recited cell types. Thus, enablement under 35 U.S.C. §112, first paragraph does not require that every amino acid of a given protein, such as an antibody, be analyzed so that the skilled artisan knows or is able to predict with absolute certainty the effect of a substitution or deletion *a priori*. Consequently, there is no need to "predict" in advance antibody variants and fragments of SEQ ID NO:1 and SEQ ID NO:3 that bind to the recited cell types in order to obtain antibody variants and functional fragments of SEQ ID NO:1 and SEQ ID NO:3. In view of the foregoing, the skilled artisan could produce antibody variants and functional fragments of SEQ ID NO:1 and SEQ ID without knowing in advance the effect of particular substitutions or deletions on activity NO:3

Finally, the number of antibody variants and functional fragments encompassed by claims 1, 2, 4, 7 to 16 and 54 to 63 are limited as they are required to have at least some degree of binding to the recited cell lines and therefore do not include inoperative embodiments. The antibodies and functional fragments of claims 1, 2, 4, 7 to 16 and 54 to 62 are further limited in number because of the high degree of sequence identity, namely they have a sequence at least 85% identical to the amino acid sequence of SEQ ID NO:1 and a sequence at least 85% identical to the amino acid sequence of SEQ ID NO:3. Moreover, because the antibodies and functional fragments encompassed by claims 1, 2, 4, 7 to 16 and 54 to 63 are required to have at least some degree of binding to the recited cell lines it is clear that there will be three CDRs regions with flanking FR regions in each of the heavy and light chain sequences. Thus, the number of antibody variants and functional fragments encompassed by the claims will necessarily be limited based upon the structural and functional requirements of antibodies discussed above (e.g., three CDRs regions with flanking FR regions in each heavy and light chain sequence),the high degree of sequence identity with SEQ ID NO:1 and SEQ ID NO:3, and the requirement of at least partial cell binding activity. Consequently, in view of the structural and functional requirements of antibodies, the number of antibody variants and functional fragments encompassed by the claims will be limited.

In terms of claims 9, 10 and 11, these claims, as amended, recite that the functional fragments bind to one or more of various cell types. Consequently, as the claimed functional fragments bind to one or more of various cell types, the ground for rejection is moot.

In terms of claims 64 and 65, these claims are directed to antibody heavy and light chain variable region sequences. Applicants recognize that a light chain variable region or a heavy chain variable region by itself may not bind to antigen. However, the proper standard for enablement under 35 U.S.C. §112, first paragraph is whether or not the skilled artisan could make and use the sequences of claims 64 and 65 without undue experimentation. Thus, provided that the skilled artisan could make and use SEQ ID NO:1 and SEQ ID NO:3 without undue experimentation, claims 64 and 65 are adequately enabled under 35 U.S.C. §112, first paragraph, regardless of whether SEQ ID NO:1 or SEQ ID NO:3 binds antigen.

Here, one skilled in the art could readily make and use heavy and light chain variable region sequences (SEQ ID NO:1 and SEQ ID NO:3) of claims 64 and 65 without undue experimentation. For example, as disclosed in the specification, each of SEQ ID NO:1 and SEQ ID NO:3 could be recombinantly or synthetically produced without undue experimentation (see the PCT publication, at page 21, line 16, to page 22, line 14). In particular, for example, each of SEQ ID NO:1 and SEQ ID NO:3 could be expressed in a eukaryotic or prokaryotic cell line in order to produce an antibody that contains SEQ ID NO:1 and SEQ ID NO:3 without undue experimentation, as disclosed in the specification (see the PCT publication, at page 21, lines 16-19). Methods of producing heavy and light chain sequences for antibodies by expressing such sequences were known in the art and routine at the time of the invention (see, e.g., Antibody Engineering, Roland Kontermann (Editor), Springer Lab Manuals, May 18, 2001; Recombinant Antibodies, Frank Breitling and Stefan Dübel, Wiley-Spektrum, September 30, 1999). Consequently, as SEQ ID NO:1 and SEQ ID NO:3 could be made and used without undue experimentation at the time of the invention, claims 64 and 65 are adequately enabled.

In view of the foregoing remarks, claims 1, 2, 4, 7 to 11, 14, 15 and 54 to 65 as amended are adequately enabled. Accordingly, Applicants respectfully request that the enablement rejection under 35 U.S.C. §112, first paragraph be withdrawn.

II. REJECTION UNDER 35 U.S.C. §102(a)

The rejection of claims 1, 2, 4, 7 to 16 and 54 to 65 under 35 U.S.C. §102(a) as allegedly anticipated by Brandlein *et al.* (Human Antibodies 11:107 (2002)) is respectfully traversed. According to the Patent Office, allegedly Brandlein *et al.* describe a monoclonal antibody CM-1 that binds to the same antigen that the claimed antibodies bind, and has the same structural and functional properties as those of the claimed antibodies and therefore, anticipates the claims.

Claims 1, 2, 4, 7 to 16 and 54 to 65 are not anticipated under 35 U.S.C. §102(a) in view of Brandlein *et al.* (Human Antibodies 11:107 (2002)). In this regard and in response to the Examiner's request for a translation of the priority application, submitted herewith is a certified English translation of German priority application DE 10210427.1, filed March 9, 2002. Accordingly, in view of the submission of the certified English translation of DE 10210427.1, the claims of the subject application are entitled to a March 9, 2002 priority date.

Brandlein *et al.* (Human Antibodies 11:107 (2002)) was not published prior to March 9, 2002. In support of Applicants' position, submitted herewith is a copy of an email received from Ms. Susan Hendriks, Marketing Coordinator at IOS Press, the publisher of Human Antibodies. In the email Ms. Hendriks states that "Volume 11, number 4 of Human An[it]tibodies was published on April 18th 2003." Consequently, as Brandlein *et al.* (Human Antibodies 11:107 (2002)) was not published prior to March 9, 2002, it is not available as prior art.

In view of the foregoing, claims 1, 2, 4, 7 to 16 and 54 to 65 are not anticipated by Brandlein *et al.* (Human Antibodies 11:107 (2002)). Consequently, Applicants respectfully request that the rejection under 35 U.S.C. §102(a) be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 1, 2, 4, 7 to 16 and 54 to 65 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 509-4065.

Please charge any additional fees, or make any credits, to Deposit Account No. 50-2212.

Respectfully submitted,

PILLSBURY WINTHROP SHAW PITTMAN LLP

Date: September 19, 2007

Robert M. Bedgood, Ph.D.
Reg. No. 43,488

Customer No. 25700
12255 El Camino Real, Suite 300
San Diego, CA 92130-2006
Telephone: (858) 509-4065
Facsimile: (858) 509-4010

Bedgood, Robert M.

From: Susan Hendriks [s.hendriks@iospress.nl]
Sent: Monday, July 09, 2007 4:28 AM
To: Bedgood, Robert M.
Subject: RE: Human Antibodies volume 11, number 4, 2002

Dear Robert,

Volume 11, number 4 of Human Anitbodies was published on April 18th 2003. I hope I've been of enough assistance in this matter. If you have any further questions, please do not hesitate to contact me.

Kind regards,

Susan Hendriks
Marketing Coordinator

IOS Press
Nieuwe Hemweg 6B
1013 BG Amsterdam
The Netherlands
Tel.: +31 (0)20 688 3355
Fax: +31 (0)20 687 0039
www.iospress.nl

Van: Bedgood, Robert M. [mailto:robert.bedgood@pillsburylaw.com]
Verzonden: vrijdag 6 juli 2007 22:39
Aan: market@iospress.nl
Onderwerp: Human Antibodies volume 11, number 4, 2002

Dear Sir or Madam-
Please advise the month and date that Human Antibodies, Volume 11, Number 4, 2002 was published.
thanks you for your assistance.
regards,
Robert

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EXHIBIT A

TRANSLATOR CERTIFICATION

450 7th Ave | 6th Floor | New York, NY 10123 | Tel 212.643.8800 | Fax 212.643.0005 | www.mside.com

Morningside | Translations

I, Michael Magee, a translator fluent in the German language, on behalf of Morningside Translations, do solemnly and sincerely declare that the following is, to the best of my knowledge and belief, a true and correct translation of the document(s) listed below in a form that best reflects the intention and meaning of the original text.

MORNINGSIDE TRANSLATIONS

Michael Magee mp

Signature of Translator

Date: August 27, 2007

Description of Documents Translated:

DE 10210427A1

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19 FEDERAL REPUBLIC OF GERMANY

GERMAN PATENT AND TRADEMARK OFFICE

12 **Unexamined Patent Application**

10 **DE 102 10 427 A1**

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DE 102 10 427 A1

71 Applicant:
Müller-Hermelink, Hans Konrad, Prof. Dr., 97082 Würzburg, DE; Vollmers, Heinz, Prof. Dr., 97084 Würzburg, DE

74 Representative:
Pöhner, W., Dipl.-Phys. Dr.rer.nat., Patent Attorney., 97070 Würzburg

72 Inventor:
Same as applicant

56 Documents cited in the evaluation of patentability:
DE 41 07 154 A1
Br. J. Cancer, 1990, Oct. 62 (4), pp. 595-598;
Cancer 1995, Aug. 15, 76 (4), pp. 550-558;
Cancer Res. 1989, May 1, 49 (9), pp. 2471-2476;
J. Mol. Biol. 2002, Feb. 1, 315 (5), pp. 1063-1073;
Sequence search VH CM-1, AS 1-102, www.ncbi.nlm.nih.gov/blast;

The following information is taken from documents filed by the applicant

54 Human monoclonal antibody

Described is a human monoclonal antibody containing heavy and light chain molecules, each having a region of constant composition from antibody to antibody and a region of variable composition from antibody to antibody. According to the invention, at least one variable region of the light and/or heavy chains has substantially the amino acid sequence stated in Appendix 1. The invention further relates to methods for producing the antibody, use of the antibody for combating tumors, and a pharmaceutical agent and diagnostic agent containing the antibody.

DE 102 10 427 A1

Description

[0001] The invention relates to a human monoclonal antibody containing heavy and light chain molecules, each having a region of constant composition from antibody to antibody and a region of variable composition from antibody to antibody, or a functional fragment thereof. The invention further relates to methods for producing the antibody, use of the antibody for combating tumors, and a pharmaceutical agent and a diagnostic agent which contain the antibody.

[0002] Current methods for treating cancer include surgical removal of the tumor, radiation, and chemotherapy. A significant drawback of these methods is that they are not specifically targeted to the tumor cells. For surgical removal, for example, all of the tumor may not be removed, with the result that a new tumor develops and metastases possibly form which become established in other areas of the body. In the treatment of tumors by radiation or chemotherapeutic agents, the lack of selectivity often results in damage to healthy cells as well due to the agents used. The adverse result is that the doses of radiation or chemically active substances cannot be selected to be high enough that all the cancer cells are destroyed. A considerable portion of current cancer research is therefore devoted to finding more effective and in particular selectively active methods and agents for treating tumors.

[0003] Immunological studies have shown that cellular and humoral activity is measurable when the immune system cannot effectively combat malignant cells. However, this activity is not sufficient to destroy the tumor cells. A promising approach to combating tumors, therefore, is to isolate antibodies originating from the patient's immune response, suitably propagate the antibodies, and use them therapeutically.

[0004] One method from the prior art which uses this approach is the hybridoma technique, which is based on in vitro harvesting of cellular hybrids obtained by cellular fusion of normal lymphocytes with myeloma cells that are capable of unlimited viability and cell division. The hybridoma cells thus produced have the characteristics of both parent cells, and therefore have the ability of lymphocytes to produce antibodies, and also have the capability of myeloma cells for unlimited cell division and thus for producing antibodies in large quantities.

[0005] Each hybrid cell resulting from the fusion produces monoclonal antibodies whose specificity is determined by the original lymphocyte cell. The hybridoma cells are propagated, and the ones which produce antibodies of the desired specificity are then selected. Cultivation of this selection and isolation thereof results in highly specifically reactive antibodies which react only with a given antigenic determinant. Monoclonal antibodies which bind specifically to antigens of tumors thus offer promising opportunities for the diagnosis and treatment of tumor cells.

[0006] Thus, there is a need for such human monoclonal antibodies for improving the methods and agents for combating cancer. The object of the present invention is to provide a human monoclonal antibody, a method for production thereof, and diagnostic and pharmaceutical agents derived from the antibody which have a high specificity for antigens of various tumors, and which are therefore well suited for tumor-specific treatment and diagnosis.

[0007] This object is achieved according to the invention by the isolation of a human monoclonal antibody in which

- at least one variable region of the light and/or heavy chains has substantially the amino acid sequence stated in Appendix 1.

[0008] From a chemical point of view, antibodies are immunoglobulin molecules. These molecules have two identical light chains and two identical heavy chains which are joined by disulfide bridges. Each of the chains has a region containing approximately 110 amino acids with a variable sequence, whereas the remainder of each chain has a region with a constant sequence. The variable regions of light and heavy chains each include multiple hypervariable regions which are responsible for binding of antigens. The specialized structure of the hypervariable regions thus determines the specific characteristics of the antibody.

[0009] Clinical tests have demonstrated that the structure of the referenced variable regions of the antibody according to the invention, depending on the given amino acid sequence, results in a high specific activity against the antigens of tumor cells under study. Since the antigens occurring on tumor cells are not present on normal cells, it is expected that the antibodies present exhibit little or no binding to normal cells.

[0010] It is essential to the invention that one of the variable regions of the heavy chains is substantially identical to the sequence according to the invention. By virtue of being substantially identical, the referenced regions predominantly match one another. The present invention encompasses minor modifications or substitutions of the chains, provided that the monoclonal antibody or the functional portion thereof maintains tumor-specific characteristics.

[0011] The majority of tumor-specific monoclonal antibodies of the prior art involve antibodies derived from mice. However, it is disadvantageous that such antibodies have very limited use, since mouse antibodies used in humans are recognized by the immune system as foreign proteins and may be neutralized before their therapeutic effect can be realized.

[0012] In contrast, the invention proceeds from human monoclonal antibodies, which do not have these limitations of use in human medicine. These antibodies contain sequences in the hypervariable chain regions which substantially correspond to those of human immunoglobulin. The antibodies, after recognition of the determinants or epitopes of the corresponding antigens, are thus able to bind in an unhindered manner to the affected cells without a defense reaction of the immune

system. When the antibodies according to the invention are coupled to diagnostic and therapeutic agents, such antibodies are thus advantageously suitable for early recognition and effective treatment of various types of tumors.

[0013] The object is achieved with regard to the production method by the fact that the human monoclonal antibody is preferably produced by the hybridoma technique. According to one feature of the invention, for this purpose B-lymphocytes are removed from a lymphatic organ, preferably the spleen or the lymph nodes, of a cancer patient. As a result of the cancer, these lymphocytes are stimulated to produce antibodies which react specifically to the antigens of the tumor cells that are present.

[0014] Each lymphocyte is fused with a myeloma cell in vitro. According to the present invention, HAB-1 heteromycloma cells and their subclones are used. The HAB-1 heteromyeloma cell is described in the literature by Falter, G et al., HAB-1, BrJCancer 62, 595-8 (1990). Similarly, subclones of the HAB-1 cell, referred to as HAB-1.X, may be used. The resulting cell clones have the same characteristics as the original B-lymphocytes for producing antibodies. The specificity of these antibodies is determined by the original lymphocyte cell. In the present case, this means that the antibodies produced by the cell clones also correspond to the antigens of the specific tumor that is present. After the cells are selected which synthesize antibodies of the desired specificity, these cells are cultivated, and each of the hybrid cells produces human monoclonal antibody in unlimited quantities.

[0015] According to one feature of the invention, in the provided method lymphocytes are removed from patients in particular with

- adenocarcinoma of the colon, pancreas, prostate, breast, esophagus, and/or the mouth and throat,
- bronchial carcinoma.

[0016] In addition to production of the present human monoclonal antibody by the hybridoma technique, the invention also encompasses other production methods. Direct synthesis by the recombinant method, known to one skilled in the art, or production using the known phage bank method (phage display) are provided, in particular for the production of small functional fragments.

[0017] Propagation is performed by use of the known polymerase chain reaction (PCR).

[0018] The PCR method is known to one skilled in the art, for example from US 4,683,195. This method is used for the targeted propagation of a specific DNA fragment, and is advantageously employed when DNA segments are present only in trace quantities. By use of the method, one known DNA sequence from a number of similar sequences may be identified, and may be propagated in vitro in large quantities in a short time. A specialized DNA sequence may be propagated by a factor of approximately 100,000 over a time period of approximately 3 h

[0019] When the present method is used for producing the monoclonal antibodies according to the invention or a functional fragment thereof, RNA of the hybridoma cells, which produce tumor-specific monoclonal antibodies, is copied in vitro into complementary double-stranded cDNA, using reverse transcriptase. The cDNA, which codes functional fragments of the variable regions of the light and heavy chains, is then propagated using PCR. The PCR products are purified, extracted, and then cloned.

[0020] The composition of the constant region of the heavy chain of an antibody determines its isotype, and establishes the effector function of the antibody. For immunoglobulin, the constant region of the heavy chains is composed of one of the sequences, referred to in the literature as μ, γ, δ, α, or ε, and the constant region of the light chains is composed of one of the sequences κ or λ. The various compositions of the heavy chains result in the five immunoglobulin classes IgA, IgD, IgE, IgG, and IgM. The antibodies according to the present invention generally belong to class IgM, and light chains of class λ and κ may be present. The antibody may also have a composition of the IgG class.

[0021] The invention encompasses monoclonal antibodies and functional fragments thereof. The functionality of the referenced fragments is characterized in that said fragments have the characteristics of the antibody. Such characteristics, for example, may be that the fragments have the ability to bind with antigens, or have specificity for tumor cells, or that the fragments have an effector function due to the composition of their constant region. According to one feature of the invention, fragments in particular are included which according to known nomenclature (e.g., Cell Biophysics, 22 (1993), pp. 189–224) belong to one of the groups

V_L, V_H, Fv, Fc, Fab, Fab', $F(ab')_2$.

The V_L group comprises fragments which include the variable region, or the variable and constant region, of the light chains;

the V_H group comprises fragments which include the variable region, or the variable and constant region, of the heavy chains;

the Fv group comprises fragments which include the variable regions of the heavy and light chains or portions thereof;

the Fc group comprises fragments which include the constant regions of the heavy chains or portions thereof;

the Fab group comprises fragments which are larger than the fragments of the Fv group;

the Fab' group comprises fragments which are larger than the fragments of the Fab group;

the $F(ab')_2$ group comprises fragments which contain the variable regions of both heavy and both light chains or portions thereof, and which optionally contain the first constant regions of both heavy chains or portions thereof.

[0022] Specialized requirements for given applications may be met by use of the referenced fragments. According to one feature of the invention, the characteristics of the antibody or the functional fragments thereof may be modified by substituting and/or inserting and/or removing individual amino acid groups. These types of approaches may be used, for example, to modify the stability or the selectivity of the antibody or the functional fragments thereof while maintaining the global characteristics of the antibody, for example the ability to bind to tumor antigens.

[0023] According to the present invention, the antibodies or the functional fragments thereof may be joined to additional active substances. The fields of application for the present antibody may be significantly expanded by coupling of such substances. In particular, the human monoclonal antibody according to the present invention may be used for diagnostic methods for detecting tumor cells, and for therapeutic methods for combating tumor cells.

[0024] According to one feature of the invention, the following substances in particular are provided:

- a radioactive substance,
- and/or a dye,
- and/or an enzyme,
- and/or an immunotoxin,
- and/or a growth inhibitor,

whereby these active substances may be used for

- qualitative or quantitative detection,
- decreasing proliferation,
- producing apoptosis, or
- avoiding metastasis formation

of tumor cells.

[0025] The detection of tumor cells is frequently carried out using methods known by those skilled in the art as immunoassays, which are based on the antigen-antibody reaction. To obtain quantitative information from this reaction, the antibody according to the present invention is coupled to an easily detectable labeling substance. The substance and the coupling are selected such that the immunological characteristics of the components are substantially maintained. The most well-known immunoassays are the radioimmunoassay, enzyme immunoassay, and fluorescence immunoassay. The diagnostic substances coupled to the antibodies allow sensitive and reliable methods for early detection of cancer.

[0026] The purpose of the referenced cytotoxic substances is to reduce the viability or the division capability of the tumor cells. Alternatively, the substances suppress DNA synthesis or cell division, or cause apoptosis of the cells or nonapoptotic cell death, and thus terminate growth of tumor cells or kill such cells.

[0027] By coupling the referenced substances with antibodies according to the present invention that are selectively active against cancer cells, it is possible to combat various types of tumors in a targeted and effective manner. The invention pro

- diagnosis
- and/or prevention
- and/or treatment

of the following tumors:

- adenocarcinoma of the colon
- large cell bronchial carcinoma
- ductal carcinoma of the breast
- adenocarcinoma of the endometrium
- teratocarcinoma.

[0028] Lastly, the present invention also encompasses a pharmaceutical agent and a diagnostic agent, characterized in that the active substances thereof contain the referenced monoclonal antibody or functional fragments thereof. The referenced agents generally contain further additives, such as physiological solutions, solvents, glycols, oils, or similar substances known from the prior art.

METHODS, EXAMPLES, AND PARTICULARS

CM-1 antibody

Heavy chain (VH)

IGHV3-30/3-30.5 · 01 = VH region of homologous germ line gene 3-30/3-30.5 · 01
IGHJ5 · 01 = IH region of homologous germ line gene IGHJ5 · 01

Amino acid sequence

See Appendix 1

DNA sequence

See Appendix 1

Light chain (VL)

IGLV3-25 · 03 = VL region of homologous germ line gene IGLV3-25 · 03
IGLJ3 · 01 = IL region of homologous germ line gene IGLJ3 · 01

Amino acid sequence

See Appendix 1

DNA sequence

See Appendix 1

Method 1:
Immortalization of lymphocytes and primary testing of antibodies

[0029] For immortalization, the lymphocytes were fused with a variant of HAB-1 heteromyeloma according to standard protocol and cultivated. In summary, lymphocytes were fused with HAB-1 cells by means of PEG. The trioma were seeded on four 24-hole plates. The average growth rate was 80-90%, with 50% of the grown clones secreting immunoglobulins.

[0030] The first testing of the secreted human monoclonal antibodies was carried out using ELISA to determine the isotype. The next test was an immunohistochemical staining on cryosections of the autologous tumor.

Media required:

RPMI 1640 (PAA) without additives;
RPMI 1640 containing HAT supplement (HAT supplement, PAA) and 10% FCS, 1% glutamine, and 1% penicillin/streptomycin.

Immortalization:

Wash HAB-1 (fusion partner) twice with RPMI without additives;
Centrifuge for 5 min at 1500 rpm;
Thaw frozen lymphocytes (from spleen or lymph nodes) and wash twice with RPMI without additives, and likewise centrifuge;
Take up each of the two pellets in 10 mL RPMI without additives, and count in the Neubauer counting chamber;
Fuse in an HAB-1 to lymphocyte ratio of 1:2 to 1:3;
Combine the cell pellets after the second washing, mix, and centrifuge for 8 min at 1500 rpm;
Carefully add the PEG (polyethylene glycol 1500, Roche), previously warmed to 37°C, dropwise to the pellet with slight rotational motions of the 50-mL test tube;
Gently resuspend, and then allow to rotate for exactly 90 sec in a water bath at 37°C
Wash out the PEG with RPMI without additives (two full No. 10 pipettes)
Centrifuge for 5 min at 1500 rpm;
Plate 24-well plates with 1 mL RPMI containing HAT supplement
Dissolve the pellet in RPMI containing HAT supplement;
Pipette 0.5 mL of the cells into each of the 24 wells;
Place fusion plates in drying oven;
Replace medium weekly with RPMI containing HAT supplement.

Method 2:
Molecular characterization of the antibodies

[0031] For sequencing of the monoclonal antibodies, cDNA was produced from total RNA (RNAse kit, Quiagen) from trioma (M-MLV reverse transcriptase, Gibco). The corresponding VH genes were then propagated by PCR amplification (Taq polymerase, MBI Fermentas). The PCR products were purified by gel electrophoresis and extracted. After cloning of the PCR products (pCR-Script Amp SK + cloning kit, Stratagene), the positive closes were sequenced (DyeDeoxy termination cycle sequencing kit, Applied BioSystems). The sequences were analyzed using DNASIS for Windows, Genebank, and V-Base databases (Vollmers et al., 1998).

Immunohistochemical characterization

[0032] Antibodies which react with the autologous tumor were tested on a panel of normal tissues and tumorous tissues in the immunoperoxidase test (see below for protocol) in order to obtain an overview of the antibody reaction and the antigen distribution.

[0033] Antibodies which react specifically with the tumor cells but not with healthy tissue were tested further, first against the same types of tumors from different patients, then against tumors from other organs, and finally against normal tissue. More detailed characterization of the antibody and antigen is not possible unless the reaction pattern of the antibody supports a conclusion of at least limited specificity for malignant tissue.

Immunoperoxidase staining on cryosections and cytospins

Slides;
Allow slides to dry for at least 2 h after cutting;
Place slides in acetone for 10 min;
Allow to dry for 30 min;
Wash 3x with Tris-NaCl and then allow to stand in Tris-NaCl for 5 min;
Saturate with 100 µL milk powder (3% in PBS) for 15–30 min;
Wash 3x with Tris-NaCl;
100 µL of the respective 1st antibody:
for negative control, with RPMI:
for positive control, 1:50 CK8 with BSA/PBS or 1:10 CAM 5.2 with BSA/PBS (0.5% BSA in PBS);
Incubate for 30 min;
Wash 3x with Tris-NaCl;
100 µL of the respective 2nd antibody:
70% conjugated rabbit anti-mouse peroxidase with PBS + 30% human serum + 1:50 antibody;
70% conjugated rabbit anti-human IgM peroxidase with PBS + 30% rabbit serum + 1:50 antibody;
Incubate for 30 min;
Wash 3x with Tris-NaCl;
Place slides in PBS for 10 min;
Dissolve 1 DAB tablet and 1 H_2O_2 tablet in 1 mL tap water;
Pipette 100 µL substrate onto the slides and incubate for 10 min;
Rinse with distilled water;
Place slides in hemalum for 5 min;
Rinse with water for 15 min;
Place slides in distilled water and mount with glycerin gelatin.

Staining of colorectal tissues

[0034] Colorectal tissues were stained to evaluate the number of different carcinomas, and the intensities, to which the antibodies being studied showed a reaction. A total of 21 tumors were available for this purpose, of which 10 (47.6%) came from female patients and 11 (52.4%) came from male patients. Of the 21 preparations, 20 were adenocarcinomas, of which 2 also contained signet ring cells, and for which a tumor had the histological characteristics of a squamous cell carcinoma, consisting of 3 carcinomas of the caecum, 2 of the colon sigmoideum, 5 of the rectum, and 11 in the remaining areas of the colon.

Antibody	stained	–	+	++	%
CM-1	21	2	11	8	90.5

– Negative result
\+ Mild to moderately positive staining
++ Strongly positive staining

Cell Death ELISA^{PLUS} (Roche, Mannheim)

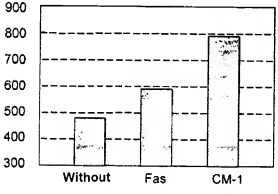
[0035] The extent of apoptosis induction by CM-1 antibody was analyzed using Cell Death Detection ELISA^{PLUS}. This test is based on the principle of a quantitative sandwich enzyme immunoassay, using the peroxidase-conjugated mouse monoclonal antibodies directed against histone or DNA components. Following enzymatic conversion of a colorless substrate, the quantity of nucleosomes, and thus the relative number of apoptotic cells, present may be photometrically determined on the basis of the color intensity of the reaction product.

[0036] For this purpose, 100 µL of a cell suspension (1.0×10^5/mL) of the various cell lines together with 100 µL of the undiluted or 1:1 diluted antibody supernatants in a 96-well plate were incubated for 24 h in a drying oven at 37°C and 7%

CO_2. After completion of incubation the cells were centrifuged for 10 min at 200 g, the supernatant was aspirated, and 200 µL lysis buffer was added, resulting in cell lysis over the following 30 min at room temperature. After recentrifugation, 20 µL of the supernatant was transferred to each streptavidin-coated microtiter plate, and 80 µL of the immunoreagent (1/20 anti-DNA-POD, 1/20 anti-histone biotin, 18/20 incubation buffer) was then added by pipette. In addition, a positive control contained in the test kit and a blank assay were performed. After thorough mixing of the plates for 2 hours at approximately 250 rpm, followed by washing three times with incubation buffer (250 µL), 100 µL of the ABTS solution (1 ABTS tablet in 5 mL substrate buffer) was pipetted into each well. After remixing, the intensity of the antibody-induced apoptosis was indicated by an intense green precipitate. The color intensity was measured using an ELISA reader at λ = 415 nm against a reference wavelength of 490 nm, and on this basis the intensity of the antibody-induced apoptosis was calculated.

Cell Death ELISA

Antibody: CM-1
Cell line: CACO-2
Incubation time: 24 h



Without:	Negative control (RPMI 1460 medium)	
CD95 Fas:	2 µg/ML	Positive control (commercially available antibody
CM-1	45 µg/mL	Antibody supernatant

[0037] After incubation for 24 hours, the CM-1 antibody being studied showed pronounced apoptosis indication [sic; induction] compared to the negative controls, the effect for CM-1 exceeding that of the negative control by a factor of 1.46.

MTT test

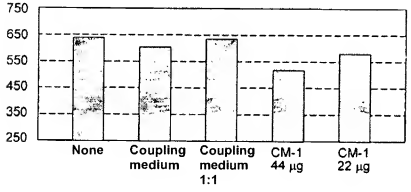
Trypsinize cells and resuspend in 10 mL RPMI complete medium (RPMI 1640, 10% FCS, 1% glutamine, 1% penicillin/streptomycin);
Count cells and dilute to 1 x 10^6 cells per mL;
Pipette 50 µL cell suspension per well into a 96-well plate (leave first row empty); i.e., a cell count of 5 x 10^4 cells is present in each well;
Add 50 µL antibody (various dilutions in complete medium) per well;
Incubate the 96-well plate for 24 h or 48 h in a drying oven
Pipette 50 µL MTT solution into each well;
Incubate plate for 20 min in the drying oven;
Centrifuge plate for 10 min at 2800 rpm and aspirate supernatant;
Add 150 µL DMSO per well and resuspend the cell pellet;
Determine absorption at a wavelength of 540 nm and 690 nm, using the ELISA reader.

MTT

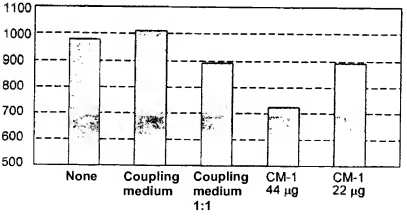
Dissolve 5 mg/mL 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium (SIGMA) in PBS.

MTT test

Antibody: CM-1
Cell line: COLO-206F (colon carcinoma)
Incubation time: 24 h



Antibody: CM-1
Cell line: COLO-206F (colon carcinoma)
Incubation time: 48 h



<110> Prof. Dr. Müller-Hermelink
Prof. Dr. Vollmers

<120> Human monoclonal antibody

<141> March 9, 2002

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 1               5                  10                  15                  20

tgg gtc cgc cag gct cca ggc aag ggg ctg gac tgg gtg gca gtt ata tca tat gat gga      120
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Val Ile Ser Tyr Asp Gly
            25                  30                  35                  40

agt aat aaa tac tat gca gac tcc gtg aag ggc cga ttc acc atc tcc aga gac aat tcc      180
Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
            45                  50                  55                  60

aag aac acg ctg tat ctg caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac      240
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
            65                  70                  75                  80

tgt gca aaa gac cgg tct tcg gga tac tac ggt atg gac gtc tgg ggc caa ggc acc ctg      300
Cys Ala Lys Asp Arg Ser Ser Gly Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Leu
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gtc acc
Val Thr                                                                          306
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<110> Prof. Dr. Müller-Hermelink
Prof. Dr. Vollmers

<120> Human monoclonal antibody

<141> March 9, 2002

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 1               5                  10                  15                  20

acc tgc tct gga gat gca ttg cca aag caa tat gct tat tgg tac cag cag aag cca ggc   120
Thr Cys Ser Gly Asp Ala Leu Pro Lys Gln Tyr Ala Tyr Trp Tyr Gln Gln Lys Pro Gly
            25                  30                  35                  40

cag gcc cct gtg ctg gtg ata tat aaa gac agt gag agg ccc tca ggg atc cct gag cga   180
Gln Ala Pro Val Leu Val Ile Tyr Lys Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg
            45                  50                  55                  60

ttc tct ggc tcc agc tca ggg aca acg gtc acg ttg acc atc agt gga gtc cag gca gaa   240
Phe Ser Gly Ser Ser Ser Gly Thr Thr Val Thr Leu Thr Ile Ser Gly Val Gln Ala Glu
            65                  70                  75                  80

gac gag gct gac tat tac tgt caa tca gca gac agc agt ggt act tat gtg gta ttc ggc   300
Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ala Asp Ser Ser Gly Thr Tyr Val Val Phe Gly
            85                  90                  95                  100

gga ggg acc aag ctg acc gtc cta ggt                                              327
Gly Gly Thr Lys Leu Thr Val Leu Gly
            105
```

Claims

1. Human monoclonal antibody containing heavy and light chain molecules, each having a region of constant composition from antibody to antibody and a region of variable composition from antibody to antibody, or a functional fragment thereof, **characterized in that** at least one variable region of the light and/or heavy chains has substantially the amino acid sequence stated in Appendix 1.
2. Method for producing the human monoclonal antibody or a fragment thereof according to Claim 1 using the hybridoma technique, characterized in that
the hybridoma cells are obtained by fusion
of HAB-1 heteromyeloma cells and their subclones
with B-lymphocytes removed from
a lymphatic organ, preferably the spleen or the lymph nodes, of a cancer patient.
3. Method for producing the human monoclonal antibody or a fragment thereof according to Claim 2, characterized in that the B-lymphocytes are removed from a patient with
adenocarcinoma of the colon, pancreas, prostate, breast, esophagus, and/or the mouth and throat,
or bronchial carcinoma.
4. Method for producing the human monoclonal antibody or a fragment thereof according to Claim 1, characterized in that the human monoclonal antibody or fragment thereof is produced using the recombinant method.
5. Method for producing the human monoclonal antibody or a fragment thereof according to Claim 1, characterized in that the human monoclonal antibody or fragment thereof is produced by gene technology, using phage banks (phage display method).

6. Human monoclonal antibody or a functional fragment thereof according to one of Claims 1–5, characterized in that the structure of the constant region of the heavy chains corresponds to immunoglobulin M or G (IgM or IgG).
7. Human monoclonal antibody or functional fragment thereof according to one of Claims 1–6, characterized in that the referenced functional fragment belongs to one of the groups
- V_L
- V_H
- Fv
- Fc
- Fab
- Fab'
- $F(ab')_2$.

8. Human monoclonal antibody or functional fragment thereof according to one of Claims 1–7, characterized in that individual amino acid groups are
substituted,
and/or inserted,
and/or removed.
9. Human monoclonal antibody or functional fragment thereof according to one of Claims 1–8, characterized in that a first substance is coupled, in particular
a radioactive substance,
and/or a dye,
and/or an enzyme,
and/or an immunotoxin,
and/or a growth inhibitor.
10. Human monoclonal antibody or functional fragment thereof according to one of Claims 1–9, characterized in that a second substance is coupled, in particular for
qualitative or quantitative detection,
decreasing proliferation,
producing apoptosis, or
avoiding metastasis formation
of tumor cells.
11. Use of the monoclonal antibody or a functional fragment thereof according to one of the preceding claims for combating tumors, characterized in that the monoclonal antibody or functional fragment thereof is used for the
diagnosis
and/or prevention
and/or treatment of the following tumors in particular:
adenocarcinoma of the colon, pancreas, prostate, breast, esophagus, and/or the mouth and throat, or
bronchial carcinoma.
12. Pharmaceutical agent, characterized in that the active substance thereof contains the referenced monoclonal antibody or functional fragments thereof.
13. Diagnostic agent, characterized in that the active substance thereof contains the referenced monoclonal antibody or functional fragments thereof.

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